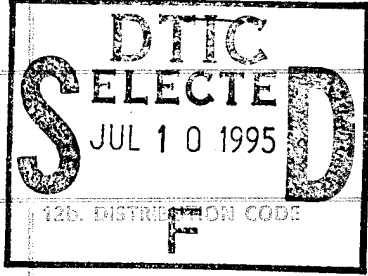


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FINAL REPORT

GRANT#: N00014-89-J-1808

R&T Code: 4415911

PRINCIPAL INVESTIGATOR: Dr. Louis Marzella

INSTITUTION: University of Maryland School of Medicine

GRANT TITLE: Effect of Pressure on Intravascular Adhesion Molecules

AWARD PERIOD: 1 May, 1989 - 30 April, 1995

OBJECTIVE: 1) To elucidate the pathophysiology of spinal cord injury induced by decompression trauma 2) To develop a rapid and quantitative assay in an animal model of decompression trauma to quantify the cord injury and permit screening of pharmacologic interventions designed to decrease or block the injury and enhance recovery.

APPROACH: We developed the animal model by subjecting rats to simulated dives using compressed air in a hyperbaric chamber. We then tested techniques for the quantification of spinal cord injury using functional analysis of gait and electrophysiologic measurements of evoked potentials. For this purpose, evoked action potentials were recorded from the plantaris muscle after stimulation of the sciatic nerve at the sciatic notch with a needle electrode. Both the motor (M) and reflex (H) responses were recorded. The following parameters were measured: latency, maximal amplitude, and magnitude of the responses. We then assessed the role played by ischemia in injury to the spinal cord injury and brain and in various other organs affected by decompression sickness. To this end, organ blood flows were measured with radiolabeled microspheres (15 μ diameter). Cardiac outputs were calculated by the reference organ technique. The role of extravascular gas in cord injury was assessed by morphometric analysis of spinal cord sections. The spinal cords were fixed by perfusion-fixation and multiple sections from cervical, thoracic, and lumbar spine were obtained. After paraffin embedding, some sections were stained with lectin-HRP to identify capillaries. Other sections were stained with hematoxylin and eosin and Luxol fast blue to aid in the evaluation of myelin sheaths. The white matter and space occupying lesions judged to represent bubbles were quantified in a blinded fashion with a digitizer tablet. We finally looked for expression of adhesion molecules, recruitment of leukocytes and activation of endogenous effector cells in the cord following decompression trauma. Immunostaining techniques were used to evaluate the expression of leukocyte adhesion molecules (β_2 -integrins) and their endothelial cell counter receptor (intercellular adhesion molecule-1), as well as markers of macrophage (ED-1) and glial cells (OX-42 and GFAP). To this end, cryosections of spinal cord were incubated with primary antibody followed by biotinylated anti IgG antibody and an avidin-biotin-horseradish peroxidase complex. The antigen was visualized by the addition of diaminobenzidine and H_2O_2 . Leukocyte surface expression of β_2 -integrins was also determined by immunostaining of circulating leukocytes followed by analysis by flow cytometry.

ACCOMPLISHMENTS:

DEVELOPMENT OF ANIMAL MODEL

The animal model was adopted from the work of Lillo and coworkers at the Naval Medical Research Institute. Rats were subjected to a simulated dive in a steel hyperbaric chamber using compressed air. Ten different diving profiles were tested. Rats subjected to diving-profile 1 were exposed to a pressure equivalent to 165 feet of sea water. The rats were compressed and decompressed at 0.27 ft/sec with a 10 min stop at

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100 ft during decompression. Total bottom time was 90 min. No evidence of decompression sickness was noted in these animals and they were used as controls. In diving profiles 8, 9 and 10, the rats were compressed to a depth of 198 feet of sea water for a bottom time of 70 min duration. Decompression was performed at rates of 3.3, 6.6, or 9.9 ft/sec respectively. These animals showed signs of decompression sickness and mortality ranged from less than 10% in the milder protocol to approximately 80% in the most severe protocol. Diving profile 1 was used for controls, diving profile 8 was used for the hemodynamic, immunocytochemical, and electrophysiologic studies. Diving profile 10 was used for the quantification of free gas in the cord. The rats were studied before, and at various time-points (ranging from 10 min to 5 days), after diving.

VALIDATION OF THE MODEL OF DECOMPRESSION SICKNESS; UTILITY OF ELECTROPHYSIOLOGIC MEASUREMENTS OF SPINAL CORD FUNCTION

Functional assays of motor function were attempted first. However, neither the inclined plane method nor the gait analysis method were sensitive enough to detect spinal cord injury. However, by electrophysiologic measurements in the rat lower limbs, a significant and complex pattern of neurologic dysfunction was demonstrated in the animals subjected to diving. Analysis of evoked potentials in the plantaris muscle showed that the sensory reflex response was markedly depressed within 1 hr after diving and remained depressed for at least 5 days after diving. The motor response was also depressed soon after diving, although to a lesser degree, and improved over time. The latency of the responses did not appear to be affected by diving in the early time points. However, 3 days after diving, prolongation of the latency was observed. These results indicate that the locus of the paresis induced by diving is of central (spinal) origin. These electrophysiologic data establish the validity of our rat model for the study of spinal cord injury. These measurements also provide a rapid, relatively-non-invasive means of quantifying the injury.

MECHANISMS OF SPINAL CORD INJURY

1. Role of Ischemia

The role of ischemia in decompression-induced spinal cord injury was evaluated by measuring blood flow to the central nervous system and other organs. Control rats were subjected to diving profile 1, experimental rats to profile 8. The rats were studied before and at 10 min, 1 hr, and 3 hr after diving. No significant changes were observed in mean blood pressure (85 mm Hg), heart rate (462 beats/min), or cardiac output index (35.9 ml/min 100g body weight) in either controls or experimental animals after diving. The control animals also showed no changes in blood flows to any of the organs examined. In the experimental animals, there was no evidence of decrease in blood flow to any of the regions of the central nervous system examined (cervical, thoracic, and lumbar spine; cerebellum, medulla, pons, midbrain, cerebral cortex). Blood flow to the spinal cord increased significantly (20%) at 10 min after diving and returned to normal by one hr. The increased blood flow may have been in response to hypoxia or hypercapnia and was accounted for by a significant increase (60%) in the percentage of cardiac output delivered to the spinal cord. A trend toward increased blood flow and percentage of cardiac output was also observed in the brain at 10 min after diving. The following changes in blood flow were observed in the other organs examined. Decreased blood flow occurred at 10 min in lung (40%) and non-respiratory skeletal muscle (50%); the blood flow returned to normal by one hour. These changes are secondary to the accumulation of free gas in the lung microcirculation and to the stress-induced shunting of blood away from musculature. Interestingly, blood flow to the gastrointestinal tract increased beginning at 1 hr after diving and remained elevated at 3 hr. Both portal and arterial blood flow to the liver were also elevated. The cause of this altered regulation of blood flow in the GI tract is not

known. The overall significance of these hemodynamic data is that they demonstrate a lack of large scale blood flow changes in the central nervous system of rats with decompression sickness. While these studies cannot rule out the occurrence of small disturbances in distribution of microcirculatory flow, it appears highly unlikely that ischemia plays an important role in the pathophysiology of spinal cord injury in the rat model of decompression sickness.

2. Role of Autochthonous Bubbles

We evaluated spinal cord sections from animals with spinal decompression sickness to determine if space-occupying lesions are present. These lesions are thought to represent autochthonous bubbles caused by the accumulation of extravascular free gas. For these studies, a very severe diving profile was selected (profile 10). In order to preserve the lesions, perfusion fixation of the spinal cord was begun 5 min after the end of the dive with a rapidly penetrating fixative. Space-occupying lesions with localization and size similar to those described in the literature were observed in the white matter of the rat spinal cord. However, the number of lesions was much lower than that observed in other models of decompression sickness. In a trial using lectin-HRP stained preparations, we found that the number of space-occupying lesions in rats subjected to diving did not increase. In preparations stained with Luxol fast blue, the number of the space-occupying lesions was greater in rats subjected to diving. From these results we conclude that in the sections of spinal cord stained with lectin-HRP, the lesions are either not sufficiently preserved or are not adequately contrasted from the tissue background. The sections stained with Luxol-fast blue, on the other hand, appear to be more suitable for the identification of the gas bubbles in the white matter. However even in these preparations, the volume of extravascular free gas present in the cord of rats with spinal decompression sickness is small. These morphologic studies indicate that the contribution of free gas in the extravascular space to the cord injury observed in the rat is minor.

3. Role of Leukocyte and Endogenous Effector Cell Activation

The role of the inflammatory reaction in spinal cord injury induced by decompression sickness is not known. In general, trauma to the central nervous system, particularly if associated with injury to blood vessels, causes the rapid recruitment of neutrophils and monocytes to the site of injury. In other types of central nervous system injury, there is a relatively low influx of neutrophils and the recruitment of monocytes occurs somewhat later. In all cases, prominent activation of endogenous effector cells, namely, microglia and astrocytes occurs. There is some evidence that by blocking the influx of leukocytes, damage to the spinal cord may be lessened. In this series of experiments, the inflammatory reaction in spinal decompression trauma was characterized. Rats were subjected to diving profile 8 and were studied beginning at 1 hr and for up to 4 days after diving. The expression of leukocyte β_2 -integrins (LFA-1 α and LFA-1 β) and their endothelial cell counter receptor ICAM-1, and of macrophage and glial markers in the cord was assessed. Brain and cord tissues from rats with experimental allergic encephalomyelitis and measles infection were used as positive controls to demonstrate the ability of the antibodies to identify leukocytes and effector cell activation. The most significant finding in these experiments was the lack of leukocyte recruitment to the cord of rats subjected to diving. This finding was validated by a lack of increased surface expression of β_2 -integrins in circulating neutrophils, monocytes, and lymphocytes. The morphology, numbers, and localization of resident macrophages, microglia, and astrocytes was also not affected by diving. Of interest, the intensity of staining and the numbers of vessels stained by anti-ICAM-1 antibodies increased between 4 to 12 hr after diving and declined after 72 hr. The lack of detectable inflammatory reaction supports the notion that extensive ischemic injury or tissue trauma does not play a major role in the cord injury in rats

with decompression sickness. In contrast with the lack of leukocyte and CNS effector cell reaction, evidence of endothelial cell activation (increased ICAM-1 immunostaining) was found in the rats subjected to diving. ICAM-1 is inducible in several cell types by a number of cytokines. In the central nervous system, increased ICAM-1 immunostaining has been found in areas of necrosis and inflammation. In the rat decompression sickness model, increased ICAM-1 immunostaining appears to be functionally unrelated to the cellular inflammatory reaction. The increased immunostaining may be a consequence of sublethal injury to endothelial cells caused by intravascular free gas.

CONCLUSIONS: In summary, in the rat model of decompression sickness we have studied there is evidence of a significant and complex pattern of spinal cord injury. The precise mechanism of the injury remains elusive. Our studies to date suggest that ischemia and the inflammatory response do not play a role in the pathophysiology. Space-occupying lesions indicative of free gas (autochthonous bubbles) were observed in the white matter of rats subjected to diving. However, the number of the lesion was much lower than that reported in other animal models. Thus the quantitative significance of the autochthonous bubbles in spinal cord injury is likely to be minor. The rat spinal cord model is amenable to further investigation aimed at defining the precise loci and further elucidating the mechanisms of the injury induced by decompression sickness. In particular, the rapid neurophysiologic quantitative assay we have developed will be of great utility for testing the efficacy of adjuvant therapies for the treatment of decompression sickness.

SIGNIFICANCE: The significance of the work we have done is to help elucidate the pathophysiology of spinal cord injury and to develop an animal model and an assay that can be used to test pharmacologic interventions designed to block the development of neurological deficits and enhance recovery.

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- 8) Marzella, L., Yin, A., Myers, R.A.M., and Darlington, D.D. Hemodynamic responses to hyperoxia in rats subjected to hypotensive hemorrhage. In Review.
- 9) Marzella, L., Tehrani, M., Yin, A., and Swoveland, P. Leukocytes, microglia, and macrophages; their role in spinal cord injury following decompression sickness in rats. In Review.

FINAL REPORT

GRANT#: N00014-89-J-1808

R&T Code: 4415911

PRINCIPAL INVESTIGATOR: Dr. Louis Marzella

INSTITUTION: University of Maryland School of Medicine

GRANT TITLE: Effect of Pressure on Intravascular Adhesion
Molecules

AWARD PERIOD: 1 May, 1989 - 30 April, 1995

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